In the Claims:

Please amend the claims as follows:

1. (previously amended) A method of altering the acetylation status of at least one amino acid residue in a protein, the acetylation status consisting essentially of an NAD-dependent acetylation status comprising the step of altering the activity of a Sir2 protein.

2. (previously amended) The method of Claim 1, wherein the protein is a histone protein.

3. (original) The method of Claim 1, wherein the amino acid residue is a lysine amino acid residue.

4. (original) The method of Claim 3, wherein the lysine amino acid residue is lysine 9 and/or lysine 14 of an H3 histone protein.

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5. (original) The method of Claim 3, wherein the lysine amino acid residue is lysine 16 of an H4 histone protein.

6. (original) The method of Claim 1, wherein the alteration in NAD-dependent acetylation status is removal of an acetyl group.

7. (original) The method of Claim 1, wherein the Sir2 protein is a Sir2α protein.

8. (previously amended) The method according to Claim 7, wherein the Sir2α protein has an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 4, 9, 12, 19 and 26.

9. (original) The method according to Claim 7, wherein the Sir2α protein is encoded by the nucleic acid sequence of SEQ ID NO: 25.

10. (previously amended) The method of Claim 7, wherein the Sir2α protein is a mutant Sir2α protein selected from the group consisting of G253A, G255A, S257A, I262A, F265A, R266A, G270A, P285A, T336A, H355A, Thr-261, Iso-271, Arg-275 and Asn-345.

11. (presently amended) A method of ~~identifying~~ testing an agent ~~which alters the~~ for ability to alter activity of a SIR2 protein ~~by assessing the ability of the agent to alter the acetylation status of at least one amino acid in the protein, the acetylation status consisting essentially of an NAD dependent acetylation~~, the method comprising ~~the steps of~~:

a) combining ~~the protein~~ a substrate that comprises an acetylated amino acid side chain, ~~the~~ an isolated or recombinantly produced Sir2 protein, NAD or an NAD-like compound and ~~the~~ an agent to be tested, thereby producing a combination; and

b) ~~detecting the NAD dependent acetylation status of an amino acid in the protein in the combination~~ determining if the acetylated amino acid side chain in the substrate is deacetylated thereby testing the agent for ability to alter activity of the SIR2 protein; ~~and~~

~~c) comparing the NAD dependent acetylation status of an amino acid in the protein in combination with the NAD Dependent acetylation status of the amino acid in the absence of the agent to be tested,~~

~~wherein a difference in the NAD dependent acetylation status of the amino acid of the protein in the presence of the agent as compared with the absence of the agent indicates that the agent alters the NAD dependent acetylation status of at least one amino acid of the protein~~.

Claims 12 to 20 are canceled.

21. (presently amended) A method of ~~identifying~~ testing an agent ~~which~~ for ability to alters life span of a cell ~~by assessing the ability of the agent to alter the acetylation status of at~~

~~least one amino acid in a protein, the acetylation status consisting essentially of an NAD-dependent acetylation status, comprising the steps of~~ , the method comprising:

a) combining ~~the protein~~ a substrate that comprises an acetylated amino acid side chain, a Sir2 protein, NAD or an NAD-like compound and the agent to be tested, thereby producing a combination;

b) ~~detecting the NAD-dependent acetylation status of an amino acid in the protein in the combination~~ determining if the acetylated amino acid side chain in the substrate is deacetylated thereby testing the agent for ability to alter activity of the Sir2 protein; and

c) contacting the agent to a cell ~~comparing the NAD-dependent acetylation status of an amino acid in the protein in the combination with the acetylation status of the amino acid in the protein in the absence of the agent to be tested,~~

~~wherein a difference in the acetylation status of the amino acid of the protein in the presence of the agent as compared with the acetylation status of the amino acid of the histone protein in the absence of the agent indicates that the agent alters the life span of the cell~~.

Claims 22 to 24 are canceled.

25. (previously amended) A method of altering the acetylation status of at least one amino acid residue in a protein, the acetylation status consisting essentially of an NAD-dependent acetylation status, comprising the step of combining the protein, a Sir2 protein and NAD or an NAD-like compound.

26. (previously amended) The method of Claim 25, wherein the histone protein is selected from the group consisting of an H2B, H3 and H4 histone protein.

27. (original) The method of Claim 25, wherein the Sir2 protein is a Sir2α protein.

Claims 28-61 are withdrawn.

Claims 62-67 are canceled.

68. (new) A method of testing an agent for ability to alter activity of a protein that comprises a SIR2 core domain, the method comprising:

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced protein that comprises a SIR2 core domain, NAD, and an agent to be tested; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated, thereby testing the agent for ability to alter activity of the protein.

69. (new) The method of claim 11 or 21 wherein the determining comprises electron-spray mass spectroscopy.

70. (new) The method of claim 11 further comprising comparing deacetylation of the substrate in the presence of the agent to deacetylation of the substrate in the absence of the agent, wherein a difference in substrate deacetylation indicates that the agent alters Sir2 protein activity.

71. (new) The method of claim 11 or 21 wherein the Sir2 protein is a human Sir2 protein.

72. (new) The method of claim 11 or 21 wherein the Sir2 protein is a murine Sir2 protein.

73. (new) The method of claim 11wherein the Sir2 protein is a fusion protein.

74. (new) The method of claim 11 or 21 wherein the substrate is a peptide substrate.

75. (new) The method of claim 74 wherein the peptide substrate is diacetylated.

76. (new) The method of claim 74 wherein the peptide substrate is a fragment of a histone protein.

77. (new) The method of claim 76 wherein the peptide substrate comprises the N-terminal tail of a histone protein.

78. (new) The method of claim 77 wherein the histone protein is histone H3.

79. (new) The method of claim 78, wherein the peptide is acetylated at positions corresponding to the lysine amino acid residue is lysine 9 and/or lysine 14 of H3 histone.

80. (new) The method of claim 11 or 21 wherein the substrate is a protein.

81. (new) The method of claim 80 wherein the substrate is a nuclear protein.

82. (new) The method of claim 80 wherein the substrate is a histone protein.

83. (new) The method of Claim 82, wherein the histone protein is selected from the group consisting of an H2B, H3 and H4 histone protein.

84. (new) The method of Claim 82, wherein the histone protein is acetylated on a lysine amino acid residue.

85. (new) The method of claim 84 wherein the histone protein is histone H4 and the protein is acetylated on lysine 16 of histone H4.

86. (new) The method of claim 11 or 21 wherein the acetylated amino acid side is a lysine.

87. (new) A method of testing an agent for ability to alter activity of a human SIR2 protein, the method comprising:

a) providing a mixture comprising a peptide substrate that comprises an acetylated lysine side chain, an isolated or recombinantly produced human Sir2 protein, NAD, and an agent to be tested; and

b) determining if the acetylated amino acid side chain in the peptide substrate is deacetylated.

88. (new) A method of testing an agent for ability to alter activity of a human SIR2 protein, the method comprising:

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced human Sir2 protein, NAD, and an agent to be tested; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated, thereby testing the agent for ability to alter activity of the human SIR2 protein.

89. (new) The method of claim 11 or 21 wherein the combination comprises $MgCl_2$.

90. (new) The method of claim 11 or 21 wherein the combination comprises DTT.

91. (new) The method of claim 68, 87, or 88 wherein the mixture comprises $MgCl_2$.

92. (new) The method of claim 68, 87, or 88 wherein the mixture comprises DTT.

93. (new) The method of claim 11, 21, 87, or 88 wherein the SIR2 protein is produced in *E. coli*.

94. (new) The method of claim 11 or 21 wherein the protein is produced in *E. coli*.

95. (new) The method of claim 11, 21, 87, or 88 wherein the agent is a protein.

96. (new) The method of claim 11, 21, 87, or 88 wherein the agent is a peptide.

97. (new) The method of claim 11, 21, 87, or 88 wherein the agent is naturally occurring.

98. (new) The method of claim 11, 21, 87, or 88 wherein the agent is non-naturally occurring.

99. (new) The method of claim 11, 21, 87, or 88 wherein the agent is chemically synthesized.

100. (new) The method of claim 11 or 88 wherein the agent is a carbohydrate.

101. (new) The method of claim 11 or 88 wherein the agent is a steroid

102. (new) The method of claim 11 or 88 wherein the agent is a lipid.

103. (new) The method of claim 11 or 88 wherein the agent is an anion.

104. (new) The method of claim 11 or 88 wherein the agent is a cation.

105. (new) The method of claim 11 or 88 wherein the agent is an oligonucleotide

106. (new) The method of claim 95 wherein the agent is an antibody.

107. (new) The method of claim 21 wherein the cell is mammalian.

108. (new) The method of claim 21 wherein the cell is a zebrafish, C. elegans, or Drosophila cell.

109. (new) The method of claim 107 further comprising evaluating lifespan of the cell.

110. (new) The method of claim 107 further comprising evaluating apoptosis.

111. (new) The method of claim 107 further comprising evaluating cellular senescence.

112. (new) The method of claim 107 further comprising evaluating proliferative state of the cell.

113. (new) The method of claim 107 further comprising evaluating acetylation state of a nuclear protein in the cell.

114. (new) The method of claim 107 further comprising evaluating acetylation state of a histone protein in the cell.

115. (new) The method of claim 11, 87, or 88 further comprising administering the agent to an organism.

116. (new) The method of claim 115 further comprising evaluating lifespan of the organism.

117. (new) The method of claim 115 wherein the organism is an organism that ages normally.

118. (new) The method of claim 115 wherein the organism is an organism that ages prematurely.

119. (new) The method of claim 68 wherein the protein comprising a SIR2 core domain is a biologically active SIR2 protein.

120. (new) The method of claim 68 wherein the protein comprising a SIR2 core domain comprises a full length SIR2 amino acid sequence.

121. (new) The method of claim 11 or 107 further comprising formulating the agent with a pharmaceutically acceptable carrier to provide a pharmaceutical composition suitable for administration to an organism.

122. (new) The method of claim 121 further comprising administering the pharmaceutical composition to an organism.

123. (new) The method of claim 121 wherein the pharmaceutically acceptable carrier comprises a carbohydrate.

124. (new) The method of claim 88 wherein an antagonist is selected.

125. (new) The method of claim 11 wherein the combination comprises NAD.

126. (new) The method of claim 11 wherein the combination comprises an NAD-like compound selected from the group consisting of: NADH, NADP, NADPH, a non-hydrolyzable NAD and 1, N6-etheno NAD.

127. (new) The method of claim 11 wherein the SIR2 protein is an isolated SIR2 protein.

128. (new) A method of evaluating a library of compounds for an agent which alters the activity of a protein that comprises a SIR2 core domain, the method comprising:

for, each of a plurality of compounds from the library of compounds,

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced protein that comprises a SIR2 core domain, NAD, and a compound of the plurality; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated, thereby testing compounds from the library for ability to alter activity of the protein.

129. (new) A method of evaluating a library of compounds for an agent which alters the activity of a protein that comprises a human SIR2 core domain, the method comprising:

for, each of a plurality of compounds from the library of compounds,

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated protein that comprises a human SIR2 core domain, NAD, and a compound of the plurality; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated, thereby testing compounds from the library for ability to alter activity of the protein.

130. (new) A method of evaluating a library of compounds for an agent which alters the activity of a SIR2 protein, the method comprising:

for, each of a plurality of compounds from the library of compounds,

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced protein that comprises a SIR2 core domain, NAD, and a compound of the plurality; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated, thereby testing compounds from the library for ability to alter activity of the protein.

131. (new) The method of claim 128 wherein the library comprises proteins or peptides.

132. (new) The method of claim 128 wherein the library comprises naturally occurring compounds.

133. (new) The method of claim 128 wherein the library comprises non-naturally occurring compounds.

134. (new) The method of claim 128 wherein the library comprises chemically synthesized compounds.

135. (new) The method of claim 128 wherein the library comprises carbohydrates.

136. (new) The method of claim 128 wherein the library comprises steroids.

137. (new) The method of claim 128 wherein the library comprises lipids.

138. (new) The method of claim 128 wherein the library comprises oligonucleotides.

139. (new) The method of claim 115 wherein the organism is a mammal.

140. (new) The method of claim 139 wherein the mammal is a rodent.

141. (new) The method of claim 11 wherein the Sir2 protein is a Sir2α protein.

142. (new) The method of claim 141 wherein the Sir2α protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1, 4, 9, 12, 19 and 26.

143. (new) The method of claim 128 wherein the isolated protein comprises a full length Sir2 amino acid sequence.

144. (new) The method of claim 128 further comprising contacting one or more of the compounds from the library to a cell.

145. (new) The method of claim 144 wherein the cell is mammalian.

146. (new) The method of claim 144 wherein the cell is a zebrafish, C. elegans, or Drosophila cell.

147. (new) The method of claim 145 further comprising evaluating lifespan of the cell.

148. (new) The method of claim 145 further comprising evaluating apoptosis.

149. (new) The method of claim 145 further comprising evaluating cellular senescence.

150. (new) The method of claim 145 further comprising evaluating proliferative state of the cell.

151. (new) The method of claim 145 further comprising evaluating acetylation state of a nuclear protein in the cell.

152. (new) The method of claim 145 further comprising evaluating acetylation state of a histone protein in the cell.

153. (new) The method of claim 128 further comprising administering one or more of the compounds from the library to an organism.

154. (new) The method of claim 153 further comprising evaluating lifespan of the organism.

155. (new) A method comprising:

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, a recombinantly produced protein that comprises a SIR2 core domain, and NAD; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

156. (new) A method comprising:

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced protein that comprises a human SIR2 core domain, and NAD; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

157. (new) A method comprising:

a) providing a mixture comprising a substrate that comprises an acetylated lysine amino acid side chain, an isolated or recombinantly produced protein that comprises a human SIR2 core domain, and NAD; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

158. (new) The method of claim 156 wherein the protein is a human SIR2 protein.

159. (new) The method of claim 128, 129, 130, 152, 153, or 154 wherein the mixture comprises $MgCl_2$.

160. (new) The method of claim 128, 129, 130, or 156 wherein the mixture comprises DTT.

161. (new) The method of claim 156 wherein the protein is a fusion protein.

162. (new) The method of claim 156 or 157 wherein the protein is produced in *E. coli*.

163. (new) A method comprising:

a) combining a substrate that comprises an acetylated lysine amino acid side chain, a human SIR2 protein, and NAD; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

164. (new) A method comprising:

a) combining a substrate that comprises an acetylated amino acid side chain, an isolated protein that comprises a SIR2 core domain, and an NAD-like compound; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

165. (new). The method of claim 164, wherein the NAD-like compound is selected from the group consisting of: NADH, NADP, NADPH, a non-hydrolyzable NAD and 1, N6-etheno NAD.

166. (new). The method of claim 163 wherein the human SIR2 protein is recombinantly produced.

167. (new). The method of claim 166 wherein the human SIR2 protein is recombinantly produced in *E. coli*.

168. (new). The method of claim 163 wherein the human SIR2 protein is isolated.